

WHAT IS CLAIMED IS:

1. A flow-through assay device for detecting the presence or quantity of an analyte residing in a test sample, said flow-through assay device comprising a porous membrane in communication with detection probes, said porous membrane defining:

5 a competitive zone that contains a first capture reagent, said first capture reagent including a first binding member immobilized on said porous membrane that is complexed to a second binding member, said second binding member being capable of producing a competitive signal when contained within said

10 competitive zone; and

15 a detection zone within which a second capture reagent is immobilized that is configured to bind to said detection probes or complexes thereof to produce a first detection signal, said second capture reagent also being configured to bind to said second binding member from said competitive zone to produce a second detection signal, wherein the amount of the analyte within the test sample is determined from said competitive signal, said first detection signal, said second detection signal, or combinations thereof.

20 2. A flow-through assay device as defined in claim 1, wherein said detection probes comprise a substance selected from the group consisting of chromogens, catalysts, luminescent compounds, radioactive compounds, visual labels, liposomes, and combinations thereof.

25 3. A flow-through assay device as defined in claim 1, wherein said detection probes comprise a luminescent compound.

4. A flow-through assay device as defined in claim 1, wherein said detection probes comprise a visual label.

30 5. A flow-through assay device as defined in claim 1, wherein said detection probes are conjugated with a specific binding member selected from the group consisting of antigens, haptens, protein A or G, neutravidin, avidin, streptavidin, captavidin, antibodies, and complexes thereof.

6. A flow-through assay device as defined in claim 1, wherein said first and second binding members of said first capture reagent are selected from the group consisting of antigens, haptens, protein A or G, neutravidin, avidin, streptavidin, captavidin, antibodies, and complexes thereof.

7. A flow-through assay device as defined in claim 6, wherein said first binding member includes an antibody and said second binding member includes an antigen.

5 8. A flow-through assay device as defined in claim 1, wherein said second binding member comprises a substance selected from the group consisting of chromogens, catalysts, luminescent compounds, radioactive compounds, visual labels, liposomes, and combinations thereof.

10 9. A flow-through assay device as defined in claim 1, wherein said second binding member comprises a luminescent compound.

10 10. A flow-through assay device as defined in claim 1, wherein said second binding member comprises a visual label.

15 11. A flow-through assay device as defined in claim 1, wherein said second capture reagent is selected from the group consisting of antigens, haptens, protein A or G, neutravidin, avidin, streptavidin, captavidin, antibodies, and complexes thereof.

12. A flow-through assay device as defined in claim 1, wherein said porous membrane further defines a calibration zone that is configured to produce a calibration signal.

20 13. A flow-through assay device as defined in claim 1, wherein the amount of the analyte within the test sample is capable of being determined from one or both of the following formulae:

$$\begin{aligned} & D_1 + x, \\ & \text{when } x > 0, D_1 = D_{1\max} \end{aligned}$$

25 wherein,

$$x = C_{1\max} - C_1;$$

$C_{1\max}$ is a predetermined maximum intensity for said competitive signal;

C_1 is the intensity of said competitive signal;

D_1 is the intensity of said first detection signal; and

30 $D_{1\max}$ is a predetermined maximum intensity for said first detection signal; or

$$\begin{aligned} & D_1 + D_2, \\ & \text{when } D_2 > 0, D_1 = D_{1\max} \end{aligned}$$

35 wherein,

D_1 is the intensity of said first detection signal;

$D_{1\max}$ is a predetermined maximum intensity for said first detection signal; and

D_2 is the intensity of said second detection signal.

14. A flow-through assay device for detecting the presence or quantity of an analyte residing in a test sample, said flow-through assay device comprising a porous membrane in communication with optical detection probes conjugated with a first antibody specific for the analyte, said porous membrane defining:

5 a competitive zone that contains a second antibody immobilized on said porous membrane that is complexed to an antigen containing an optically detectable substance, said antigen being identical to or an analog of the analyte and said optically detectable substance being capable of producing a competitive signal when contained within said competitive zone; and

10 a detection zone within which a third antibody is immobilized that is configured to bind to complexes formed between the analyte and said conjugated optical detection probes to produce a first detection signal, said third antibody also being configured to bind to said antigen from said competitive zone to produce a second detection signal, wherein the amount of the analyte within the test sample 15 is determined from said competitive signal, said first detection signal, said second detection signal, or combinations thereof.

15. A flow-through assay device as defined in claim 14, wherein said optical detection probes and said optically detectable substance of said antigen each comprise a visual label.

20 16. A flow-through assay device as defined in claim 14, wherein said optical detection probes and said optically detectable substance of said antigen each comprise a luminescent compound.

25 17. A flow-through assay device as defined in claim 16, wherein said detection probes emit a signal at a different wavelength than said optically detectable substance of said antigen.

18. A flow-through assay device as defined in claim 14, wherein said porous membrane further defines a calibration zone that is configured to produce a calibration signal.

30 19. A flow-through assay device as defined in claim 14, wherein the amount of the analyte within the test sample is capable of being determined from one or both of the following formulae:

$$D_1 + x, \\ \text{when } x > 0, D_1 = D_{1max}$$

wherein,

$$x = C_{1\max} - C_1;$$

$C_{1\max}$ is a predetermined maximum intensity for said competitive signal;

C_1 is the intensity of said competitive signal;

D_1 is the intensity of said first detection signal; and

$D_{1\max}$ is a predetermined maximum intensity for said first detection signal; or

$$D_1 + D_2,$$

$$\text{when } D_2 > 0, D_1 = D_{1\max}$$

wherein,

D_1 is the intensity of said first detection signal;

$D_{1\max}$ is a predetermined maximum intensity for said first detection signal;

and

D_2 is the intensity of said second detection signal.

20. A method for detecting the presence or quantity of an analyte residing in a test sample, said method comprising:

i) providing a flow-through assay device comprising a porous membrane in communication with detection probes conjugated with a first antibody specific for the analyte, said porous membrane defining:

a) a competitive zone within which is immobilized a second antibody complexed to an antigen containing an optically detectable substance, said antigen being identical to or an analog of the analyte and said optically detectable substance being capable of producing a competitive signal when contained within said competitive zone; and

b) a detection zone within which a third antibody is immobilized that is configured to bind to complexes formed between the analyte and said conjugated optical detection probes to produce a first detection signal, said third antibody also being configured to bind to said antigen from said competitive zone to produce a second detection signal;

ii) contacting a test sample containing the analyte with said conjugated detection probes;

iii) measuring the intensity of said competitive signal at said competitive zone, and the intensity of said first and second detection signals at said detection zone; and

iv) determining the amount of the analyte within the test sample from one or both of the following formulae:

$$D_1 + x,$$

when $x > 0$, $D_1 = D_{1\max}$

wherein,

$$x = C_{1\max} - C_1;$$

5 $C_{1\max}$ is a predetermined maximum intensity for said competitive signal;

C_1 is the intensity of said competitive signal;

D_1 is the intensity of said first detection signal; and

$D_{1\max}$ is a predetermined maximum intensity for said first detection signal; or

$$D_1 + D_2,$$

10 *when $D_2 > 0$, $D_1 = D_{1\max}$*

wherein,

D_1 is the intensity of said first detection signal;

$D_{1\max}$ is a predetermined maximum intensity for said first detection signal;

15 and

D_2 is the intensity of said second detection signal.

21. A method as defined in claim 20, wherein said optical detection probes and said optically detectable substance of said antigen each comprise a visual label.

20 22. A method as defined in claim 20, wherein said optical detection probes and said optically detectable substance of said antigen each comprise a luminescent compound.

25 23. A method as defined in claim 22, wherein said detection probes emit a signal at a different wavelength than said optically detectable substance of said antigen.

24. A method as defined in claim 20, further comprising exciting said conjugated optical detection probes at said detection zone to produce said first detection signal.

30 25. A method as defined in claim 24, further comprising exciting said optically detectable substance at said competitive zone to produce said competitive signal.

26. A method as defined in claim 25, further comprising exciting said optically detectable substance at said detection zone to produce said second detection signal.

35 27. A method as defined in claim 20, wherein said porous membrane further defines a calibration zone that is configured to produce a calibration signal.

28. A method as defined in claim 27, further comprising generating a calibration curve by plotting said competitive signal and said first and second

detection signals as calibrated by said calibration signal for a plurality of predetermined analyte concentrations.